

# Mouse model of Schistosomiasis: infection with *Schistosoma mansoni* in CD-1 mice

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Schistosomiasis is a parasitic disease that affects almost 240 million worldwide. CD1 mice were infected with cercariae of *S. mansoni*, after which infection developed for 8 weeks. Tissues were processed to immuno-histological techniques. It was performed H&E staining for overall analyses, Sirius Red for fibrosis and immunohistochemistry for inflammation biomarkers. The most infected organ was the liver, fibrosis decreased with egg development and Galectin-3 (Gal3) and Interleukin 6 (IL-6) were expressed inside granulomas.

## 1 – INTRODUCTION

Schistosomiasis is a parasitic disease caused by the trematode worms of the genus *Schistosoma*. Infection and transmission occurs due to contact with infested water. This parasitic disease is prevalent in tropical and subtropical areas especially in underdevelopment countries where access to potable water is problematic, schistosomiasis affects approximately 240 million worldwide. Infection by *Schistosoma mansoni* is the leading cause of schistosomiasis in the world and it is the most prevalent parasite in humans<sup>1</sup>.

The adult worm lives within the host circulation, where they produce fertilized eggs. In the specific case of *S. mansoni*, the eggs are expelled into the environment through feces. When the eggs reach freshwater, hatch and release ciliated miracidia that will infect a snail host. In the snail, the parasite undergoes asexual replication through sporocysts stages, eventually detaching thousands of cercariae (the form infectious for mammalian hosts) into the water. After cercariae penetrate the skin of the mammalian host, the maturing larvae (schistosomula) takes 5 to 7 weeks to start to produce eggs<sup>2</sup>. The main goal of this project is the analyze of the infection impact of *S. mansoni* in the mouse model CD-1. It was performed a study of the allocation of *S. mansoni* eggs, especially in the liver.

## 2 – MATERIALS AND METHODS

8 CD-1 mice were submitted to a standard balance food and water ad libitum at National Institute of Health (Porto, Portugal) in controlled temperature (22°C ± 2°C) and humidity (55% ± 10%), with continuous air renovation and 12 hours' light/ 12 hours' dark cycles. 4 individuals were infected with 50 cercariae *Schistosoma mansoni* and infection developed for 8 weeks (acute phase). Mice were infected by member's extremities and tail immersion, respectively. The cercariae were obtained by shedding of snails infected with miracidia (Figure 1). After infection, mice were euthanized and tissue was processed to histological and immunohistochemistry techniques.

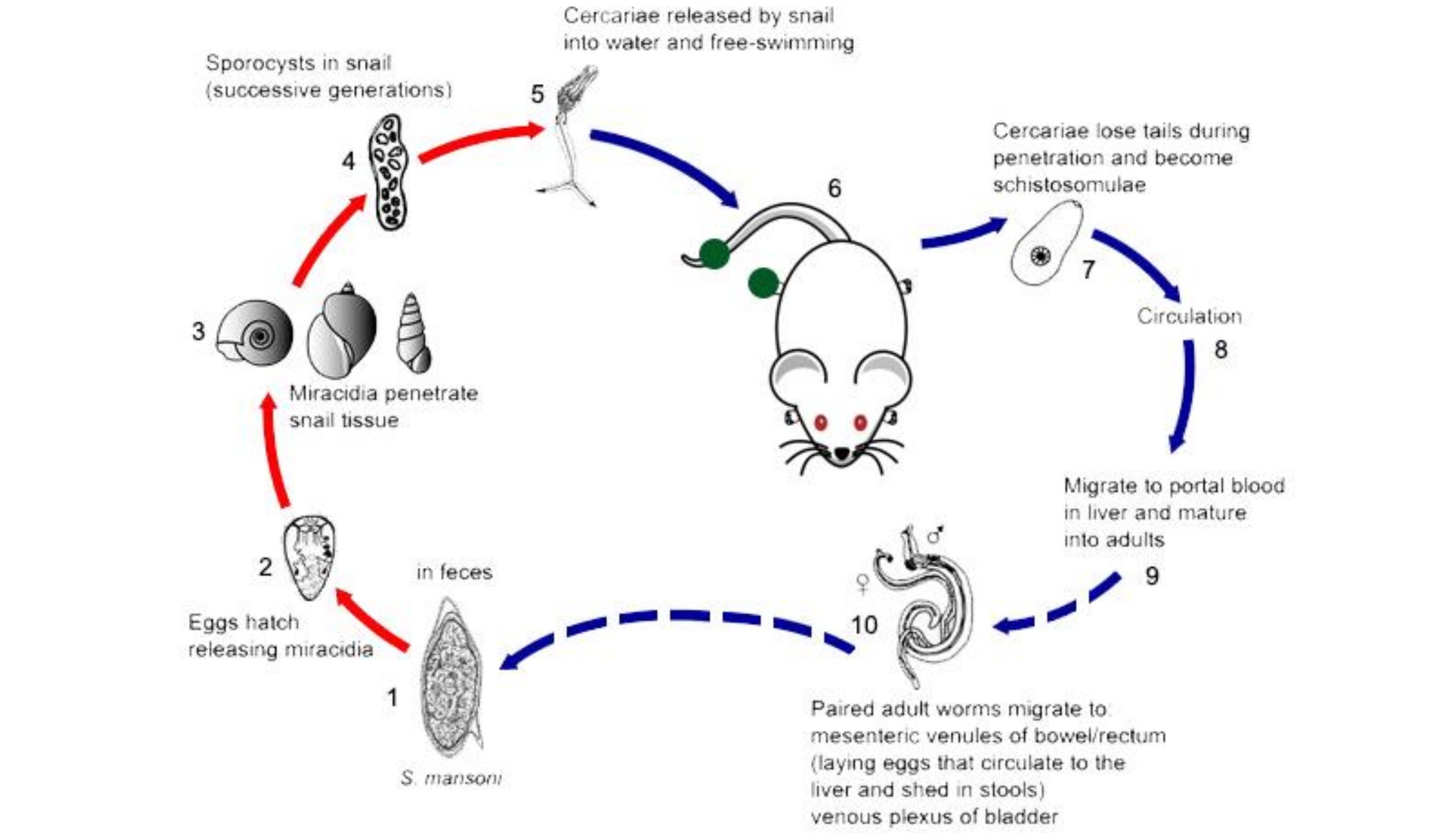


Figure 1 – Diagram illustrating infection model into CD-1 mice incorporating the *Schistosoma* life cycle from the adaptation of A. J. da Silva and M. Moser for copyright-free dissemination through the Public Health Image Library of the Centers for Disease Control and Prevention<sup>3</sup>. The stages of the *Schistosoma mansoni* life cycle include: 1. Elimination from the host as eggs in feces (diagnostic stage); 2. Hatching of miracidia; 3. Infection of species-specific aqueous snail intermediate hosts; 4. Proliferation of sporocysts within snails; 5. Release of cercariae into water (infective stage); 6. Infection of host by skin penetration; 7. Development into schistosomulae; 8. Circulation; 9. Maturation within portal vasculature, and 10. Migration of paired adult worms to target organs. Elimination of schistosoma eggs in either feces or urine depends on whether the adults reside in the mesenteric venules of the bowel/rectum. (Red arrows – Diagnostic stage; Blue arrows – Infective stage; Blue dashed arrows – Closed infected cycle although not followed on the present infective model; Green dots – Infected vias in the mouse model)

Tissues were routinely fixed in buffered formalin 10% and the embedded in paraffin. Transversal sections were executed in the microtome to performed histological techniques: H&E staining for overall analyses and Sirius Red for fibrosis identification and allowed to react with the antibodies by heat antigenic recovery accordantly with Table 1.

Table 1 – Summary table of the optimized immunohistochemistry protocols for biomarkers of interest

| Antigen    | Retrieval                         | Block                          | Primary  | Secondary  |
|------------|-----------------------------------|--------------------------------|--|--|
| CD 8       | Heat 10 min @ 98°C Extran         | 20% NSS in BSA 10% 30 min @ RT | Rabbit anti-CD8-β 1:100 (Santa Cruz, sc-19994, USA)  | Biotinylated goat anti-rabbit 1:200 (Santa Cruz, sc-2040, USA) |
| IL-6       | Heat 10 min @ 98°C Citrate buffer | 20% NSS in BSA 10% 30 min @ RT | Rabbit polyclonal anti-IL6 1:600 (Abcam, ab6672, UK) | Biotinylated goat anti-rabbit 1:200 (Santa Cruz, sc-2040, USA) |
| Galectin-3 | Heat 10 min @ 98°C Extran         | 20% NSS in BSA 10% 30 min @ RT | Rat anti-galectin-3 1:300 (eBioscience 14-5301, USA) | Biotinylated goat anti-rat 1:200 (Santa Cruz, sc-2041, USA)    |
| TNF-α      | Heat 10 min @ 98°C Extran         | 20% NSS in BSA 10% 30 min @ RT | Goat anti-TNFα 1:200 (Santa Cruz, sc-1350, USA)      | Biotinylated bovine anti-goat 1:200 (Santa Cruz, sc-2347, USA) |

## 4 – DISCUSSION AND CONCLUSION

*S. mansoni* leads to liver diseases like fibrosis and portal hypertension, it is also related to tumorigenesis due to mechanisms of inflammation and oxidative stress caused by parasite-derived molecules<sup>4,5</sup>. One of the already described cancer associated with *S. mansoni* is hepatocellular carcinoma<sup>6</sup>, for liver is the most affected organ in *Schistosoma* infection. Its egg deposition in the periportal regions lead to the production of inflammatory cytokines from macrophages that culminates in a granulomatous inflammatory reaction. These inflammatory reaction tends to decrease with the developing worm. One of these pro-inflammatory cytokine is IL6, and is secreted by macrophages in response to specific microbial molecules like parasite-derived molecules, thus justifying its expression inside granulomas. CD8+ T-cell was not observed inside granulomas, although it was previously described in other murine models (BALB/c) of *S. mansoni* infection by Pancré and colaboradores<sup>7</sup>. Previous studies demonstrated that administration of graded dosages of recombinant murine TNF-alpha to mice with chronic infection restored the size of the down modulated liver granulomas to the level of the vigorous lesions<sup>8</sup>, for the lack of the expression in the presented model supported the high levels of inflammation. Galectin 3 is a modulator of the immune/inflammatory responses during helminthic infection and is highly expressed by liver macrophages around *Schistosoma* eggs<sup>9</sup> at it was demonstrated in Figure 4c. Overall, these animal model present the main characteristics to better understand the infection implications of *S. mansoni* and a future model to improved forms of treatment.

## 5 – REFERENCES

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## 3 – RESULTS

*Schistosoma mansoni* infection led to focal areas of fibrosis around the egg deposition, accompanied by fibrosis and inflammation. Infection was observed mostly in liver (Figure 2).

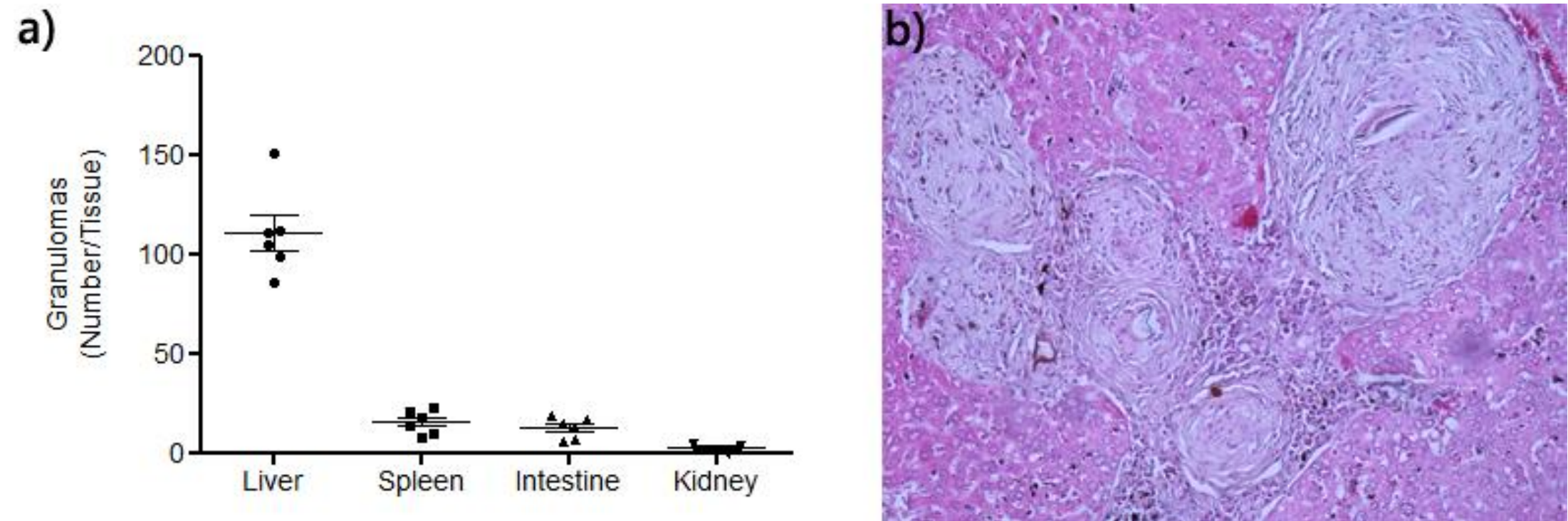


Figure 2 – a) Number of granulomas in organs. The liver was the most infected organ. In the liver the mean of granulomas is 110.7 ± 8.954, n=6 (Mean ± SEM); In the spleen 15.67 ± 2.459, n=6 (Mean ± SEM); In the intestine 12.83 ± 2.167, n=6 (Mean ± SEM) and in the kidney 2.2 ± 1.02, n=5 (Mean ± SEM). b) Representative image of H&E staining of the liver (Magnification 100x);

Sirius red staining is used to observe fibrosis levels in pathologies like schistosomiasis, it was observed that with the development of the egg into an embryonic worm, the fibrosis decreased (figure 3).

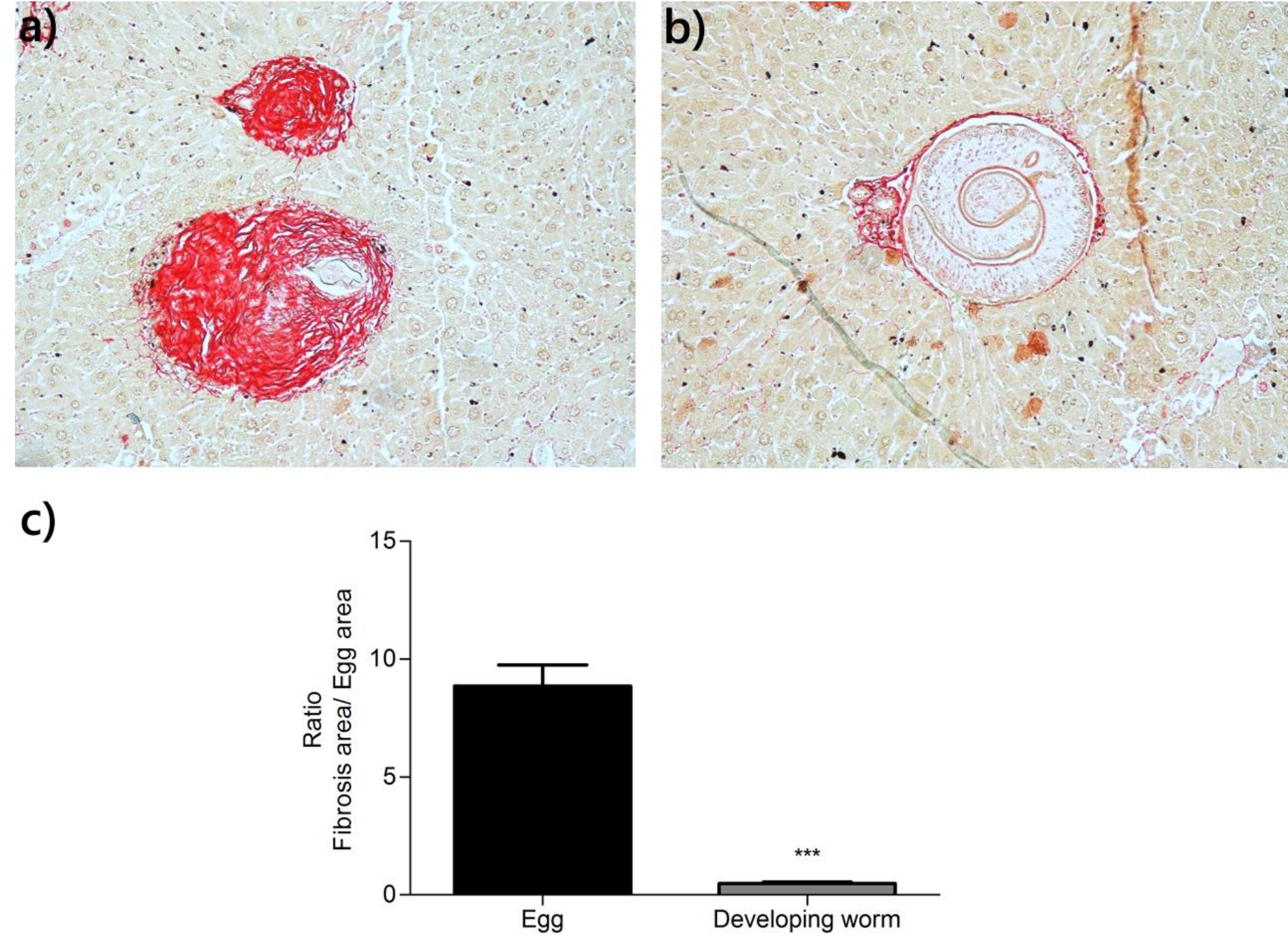


Figure 3 – Representative image of granulomas in Sirius Red staining of the liver (a) and with developing worm (b); Fibrosis is highlighted in red. (Magnification 100x); c) Significant decrease of fibrosis in granulomas with developing worm (\*\*\* p<0.001).

It was evaluated the inflammation with immunohistochemistry of inflammation biomarkers: CD 8, IL-6, Galectin-3 and TNF-α. Inside granulomas there was expression of CD-8 and Gal3 (Figure 4).

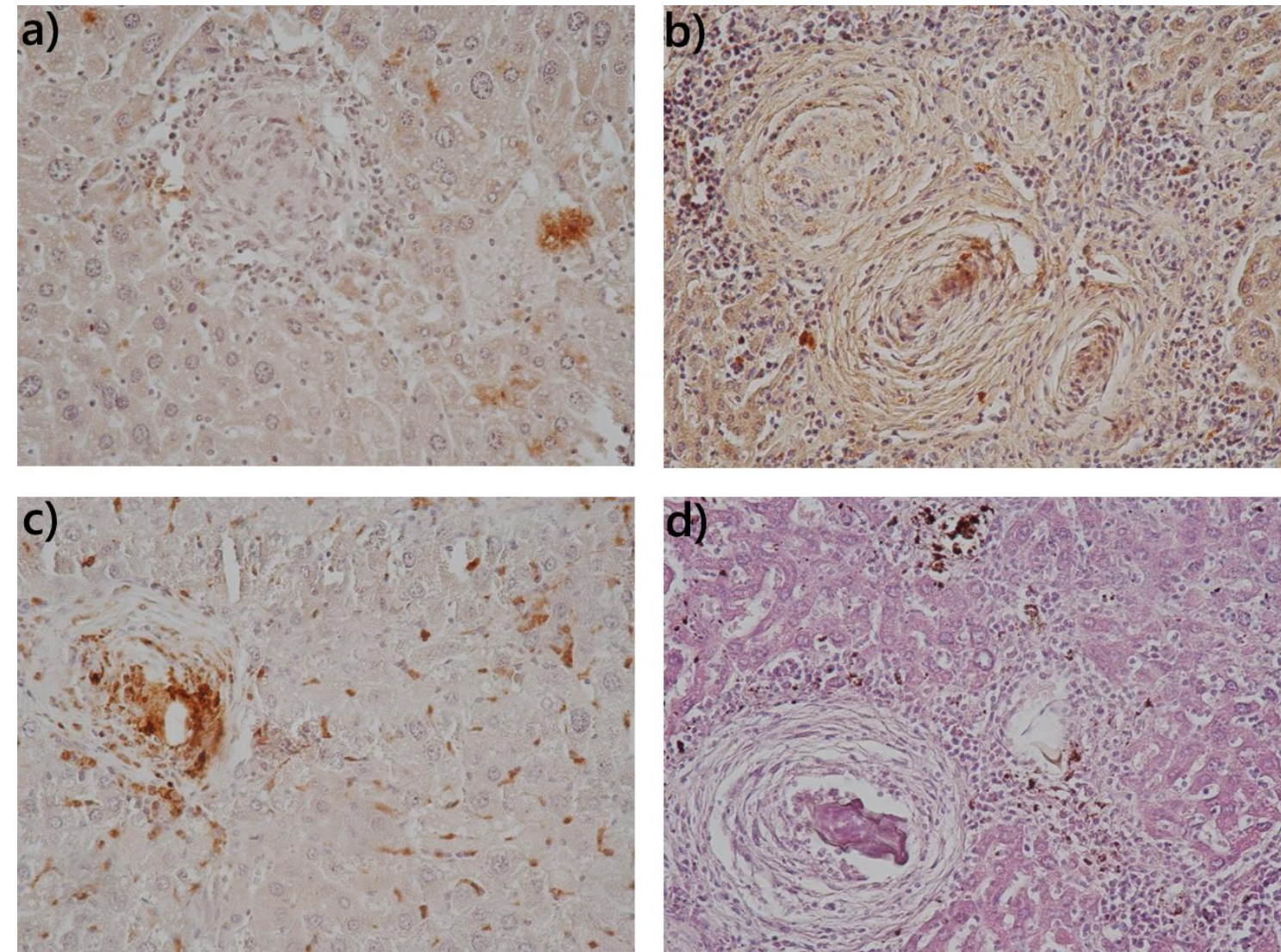


Figure 4 – Immunostaining for CD-8 (a), IL-6 (b), Galectin-3 (c) and TNF-α (d) residues in liver. Representative images are shown (Magnification 200x).

## 6 – ACKNOWLEDGEMENTS

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